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EDITORIAL REVIEW

Ontogeny of proximal tubule acidification

The proximal tubule is responsible for the reabsorption of 80% of the filtered load of bicarbonate in adults. Proximal tubule bicarbonate reabsorption is affected by a number of hormones, acid-base balance, and the extracellular fluid volume status [1]. The adult proximal tubule thus plays a central role in acid-base homeostasis. The mechanisms and regulation of proximal tubule acidification have been well characterized at the whole animal and tubular level [1]. Recent advances in cellular and molecular biology have provided further insights. However, while the neonatal proximal tubule likely also plays a critical role in acid-base homeostasis, relatively little is known about the immature segment.

The normal serum bicarbonate concentration in human infants in the first year of life averages 22 mEq/liter, and is significantly lower than that measured in adults [2]. The difference from the adult value is even more pronounced if one compares premature humans whose normal serum bicarbonate concentration averages 19 mEq/liter and where normal values can be as low as 14.5 mEq/liter [3]. The lower serum bicarbonate level in neonates and infants is due to a lower threshold for bicarbonate [2]. There are a number of potential reasons for the lower threshold for bicarbonate in neonate infants. The extracellular volume is comparatively expanded in neonates and the serum albumin is lower [4]. These variables can affect the peritubular oncotic and hydrostatic pressure to decrease net proximal tubular solute reabsorption [5–8]. There are developmental changes in diet, absorption and metabolism which may affect acid-base balance. In addition, the hormonal milieu which affects renal reabsorption of bicarbonate is significantly different in neonates [9–12]. Finally, since most of filtered bicarbonate is reabsorbed by the proximal tubule, tubular immaturity may be a significant factor to explain the maturational changes in the bicarbonate threshold. This review discusses what is currently known about the mechanism of neonatal proximal tubule acidification and the factors which may play a role in the maturation of this segment.

Neonatal proximal tubule acidification

Comparison of proximal tubule bicarbonate reabsorption in neonates and adults is not only complicated by the factors discussed above, but also by developmental nephron heterogeneity. The developing kidney displays a centrifugal pattern to nephron growth and maturation [13]. Juxtamedullary nephrons develop first and are relatively more mature at birth. In several animal species and in premature humans born before 34 weeks gestation, nephrogenesis is still occurring in the outer cortex. In the midcortex proximal tubules are at an intermediate stage of maturation [13]. This developmental heterogeneity was suggested by early bicarbonate titration studies examining the bicarbonate threshold where infants had a significant amount of splay to the titration curve, suggesting that there were populations of nephrons with various capacities for bicarbonate reabsorption [2]. Therefore, to examine if the neonatal proximal tubule has a lower rate of bicarbonate reabsorption, studies must be performed using the same nephron segment at various stages of maturation.

Schwartz et al perfused rabbit juxtamedullary proximal convoluted tubules from animals at various ages *in vitro* to directly address if there was a maturational change in the rate of bicarbonate transport (Fig. 1) [14]. The rate of bicarbonate reabsorption in neonatal proximal convoluted tubules was one-third that of the adult segment. The rate of bicarbonate transport remained fairly constant for the first three weeks of age and adult levels were measured at six weeks of age. The maturational pattern of glucose and total volume absorption was found to be similar to that of bicarbonate. While bicarbonate transport has not been directly measured in superficial nephrons, similar maturational changes have been suggested in studies in both the rat and rabbit [15–17]. Micropuncture studies have demonstrated that the rate of volume absorption in rat superficial proximal convoluted tubules measured at age 22 to 24 days was only one-half that measured in rats at 40 to 45 days [15]. In rabbit superficial proximal convoluted tubules, the maturational change in volume absorption increased fourfold in animals studied in the first week compared to those at one month of age [16]. Since in both species a significant fraction of this volume absorption is due to bicarbonate, it is likely that parallel changes in bicarbonate reabsorption were also present.

Mechanism for the lower rate of bicarbonate transport in neonatal proximal convoluted tubule

In the adult proximal convoluted tubule there is preferential reabsorption of bicarbonate over chloride ions [18, 19]. This leaves the proximal tubule with a higher luminal chloride concentration and lower bicarbonate concentration than the peritubular fluid. A lower rate of net bicarbonate transport could thus be due to a lower rate of active bicarbonate reabsorption, or a higher rate of passive backflux of bicarbonate from the peritubular capillaries into the tubular lumen. The latter would be dependent on the permeability of the paracellular pathway of the neonatal proximal tubule to bicarbonate.

There is evidence for maturational changes in the paracellular pathway which could favor a high rate of bicarbonate backflux. Urinary recovery of mannitol microinjected in proximal tubules of guinea pigs increased from 92% in neonates to 100% in adult animals, suggesting the passive reabsorption of this small molecule across the paracellular pathway only in neonates [20]. These authors also demonstrated that the length of the intercellular channels increased twofold with proximal tubule maturation to explain the relatively lower resistance of the paracellular pathway

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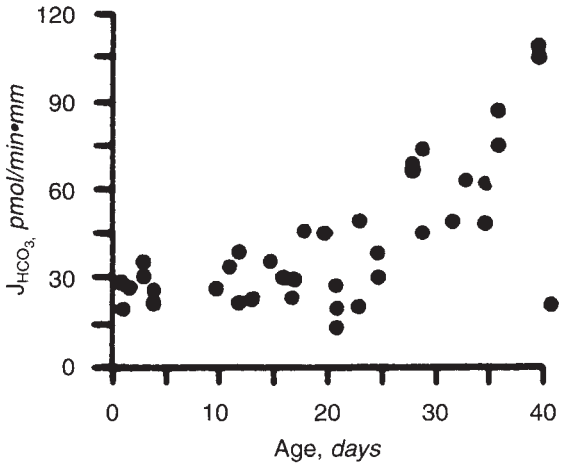

Fig. 1. *Maturational changes in the rate of juxtamedullary proximal convoluted tubule bicarbonate reabsorption (J_{HCO3}) in rabbits.* From Schwartz et al [14] with permission from the *American Journal of Physiology*.

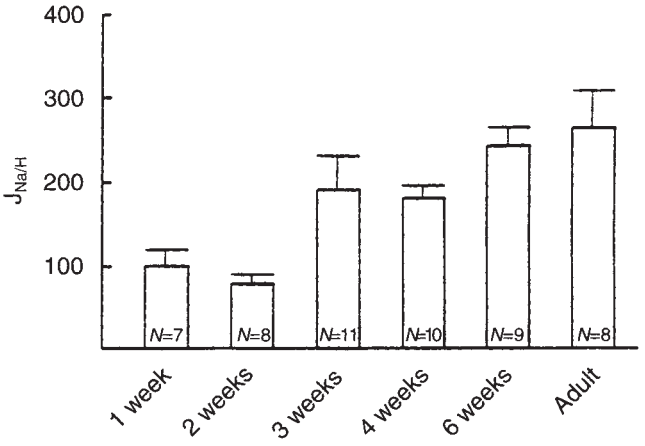

Fig. 2. *Maturational changes in the rate of juxtamedullary proximal convoluted tubule Na^+/H^+ antiporter activity in rabbits.* $J_{Na/H}$ measured as the proton flux (in pmol/mm×min) in response to luminal sodium removal. From [30] with permission from the *Journal of Clinical Investigation*.

in neonates. There was, however, no change in the length of the zonulae occludens or width of the intracellular channels [20]. Similarly, perfusion of rabbit proximal tubules at high pressure resulted in the passage of microperoxidase across intracellular spaces in neonates but not adult proximal tubules [21].

Direct measurements of bicarbonate permeability in neonatal juxtamedullary proximal convoluted tubules, however, have yielded surprising results. The bicarbonate permeability measured using isolated perfused tubules is several-fold lower in neonatal juxtamedullary proximal tubules than in adult proximal convoluted tubules [22]. Thus, the lower rate of bicarbonate transport in neonatal juxtamedullary proximal convoluted tubules cannot be explained by enhanced bicarbonate backdiffusion, but is entirely due to a lower rate of active bicarbonate transport.

In the adult proximal tubule two-thirds of apical proton secretion is mediated by the Na^+/H^+ antiporter and one-third is likely mediated by the H^+-ATPase [23, 24]. The driving force for the Na^+/H^+ exchanger is the low intracellular sodium concentration generated by the basolateral Na^+,K^+-ATPase. Bicarbonate exit is via a basolateral $Na(HCO_3)_3$ symporter [25–29]. The lower rate of neonatal proximal tubule acidification may be due to a lower rate of any of these transport processes or due to an impaired ability of the neonatal proximal tubule to generate ATP.

Several studies have demonstrated a maturational increase in Na^+/H^+ antiporter activity [30–34]. In isolated perfused rabbit juxtamedullary proximal convoluted tubules, apical Na^+/H^+ antiporter activity was measured to allow comparison to the maturational rate of bicarbonate absorption [30] (Fig. 2). The rate of Na^+/H^+ antiporter activity in one-week-old neonatal proximal convoluted tubules was approximately one-third that of the adult rate. This is the same difference found by Schwartz et al for bicarbonate transport [14]. In addition, the rate of Na^+/H^+ antiporter activity remained constant for the first two weeks of life, after which there was an abrupt increase with adult levels of Na^+/H^+ antiporter activity being measured at six weeks of age. These results again parallel that of bicarbonate reabsorption by this segment. The above study, however, solely examined juxtamedullary proximal convoluted tubules. To address if there was a difference in whole cortical Na^+/H^+ antiporter activity, Beck, Lipkowitz and Abramson studied fetal kidneys from late gestation New Zealand White rabbits [32]. Na^+/H^+ antiporter activity measured in brush border membrane vesicles from late fetal kidney cortex was one-fourth that of the adult cortex. This was entirely due to a difference in V_{max} with no change in the K_m for sodium. Therefore, there is a large maturational change in both juxtamedullary and whole cortical Na^+/H^+ antiporter activity. Maturational changes in Na^+/H^+ antiporter activity have also been studied in the rat [33, 35]. In one study rat proximal tubule cells from animals of different ages were grown in primary culture for 48 hours [33]. Na^+/H^+ antiporter activity was studied in intact cells as the amiloride sensitive sodium-dependent pH recovery from an imposed cell acidification. There was an almost twofold increase in Na^+/H^+ antiporter activity in cells from eight-day-old rats compared to cells from 40-day-old rats. One study, however, found a higher rate of Na^+/H^+ antiporter activity in neonatal rat brush border membrane vesicles compared to adults [35]. The reason for the difference between these studies and the above showing a lower rate is unknown.

There are marked changes in renal sodium balance in the transition from the fetus to the neonate which are important for the neonate's survival [36–38]. Term fetal lambs have a fractional excretion of sodium of 7% [37]. Within 24 hours of birth the fractional excretion of sodium decreases to 1% despite a concomitant threefold increase in glomerular filtration rate [37]. Recent studies have demonstrated that there is a substantial increase in Na^+/H^+ antiporter activity in the transition from the fetus to the newborn [34]. The V_{max} of the Na^+/H^+ antiporter assayed using sheep renal brush border membrane vesicles increased over twofold comparing late fetal lambs to neonates born by vaginal delivery. There was no difference in the K_m for sodium. Similar findings were also found in neonates born by cesarean section. These studies suggest that the proximal tubule Na^+/H^+ antiporter also plays a critical role in NaCl retention necessary for maintenance of salt balance and growth of the neonate.

The H^+-ATPase has been localized to the apical membrane of adult proximal tubules [39] and in the adult one-third of luminal proton secretion is likely via this transporter [23, 24]. While the

contribution of the H^+-ATPase to net bicarbonate transport has not been measured in the neonatal proximal convoluted tubule, it likely plays a very minor role. In the adult rabbit proximal convoluted tubule the Na^+/H^+ antiporter contributed to two-thirds of pH recovery from an acid load; one-third was due to the H^+-ATPase. While the rate of Na^+/H^+ antiporter activity was slower in the neonatal proximal convoluted tubule, nonetheless it was responsible for 95% of pH recovery from an acid load [31]. Only 5% of pH recovery was presumably due to the H^+-ATPase. Thus, both the Na^+/H^+ antiporter and the H^+-ATPase of neonatal proximal tubule undergo significant maturation and are factors to explain the lower bicarbonate reabsorption in neonates.

The rate of basolateral rabbit proximal convoluted tubule $Na(HCO_3)_3$ activity at one week of age was approximately 60% of the adult level [30]. Thus, the $Na(HCO_3)_3$ activity was more comparable to the adult rate than the acidification mechanisms on the apical membrane. A potential teleologic explanation for the high activity of this basolateral transporter in neonates may relate to the fact that this is the predominant transporter responsible for the defense against changes in proximal tubule cell pH in neonatal and adult proximal tubules [30, 40].

Maturational increases in renal cortical Na^+,K^+-ATPase activity have been found by several investigators [32, 41, 42]. To directly examine Na^+,K^+-ATPase activity with maturation, Schwartz et al found that the maturation of rabbit juxtamedullary proximal convoluted tubule Na^+,K^+-ATPase activity lagged behind that of volume absorption and bicarbonate transport by approximately one week [42]. This suggests that an augmentation in transepithelial solute transport or apical membrane transport may be a driving force for the maturation of the Na^+,K^+-ATPase on the basolateral membrane. Indeed, the relative amount of sodium entry via the Na^+/H^+ exchanger has been shown to affect the Na^+,K^+-ATPase activity. In proximal tubule cells in culture, an increase in Na^+/H^+ antiporter activity leads to a rise in cell sodium concentration and a secondary stimulation in Na^+,K^+-ATPase activity [43]. A similar phenomenon has been described in developing rats where chronic changes in Na^+/H^+ antiporter activity can affect Na^+,K^+-ATPase activity [44].

It appears that the rate of ATP production is not a significant variable in the maturation of proximal tubule solute transport [45]. Comparisons of the rate of oxygen consumption in neonatal and adult animals have demonstrated only small differences [46–48]. Increases in glomerular filtration rate produced by volume expansion resulted in corresponding increases in the rate of sodium reabsorption and of renal cortical oxygen consumption in neonatal rats [48]. Volume expansion in adult rats, however, did not cause significant changes in oxygen consumption. Thus, there appears to be a greater ability of neonatal proximal tubules to respond to changes in metabolic demands than the adult segment.

Carbonic anhydrase facilitates the interconversion of CO_2 and H_2O to H_2CO_3. Carbonic anhydrase is located in the cytosol and on the apical and basolateral membrane of the proximal tubule. This enzyme plays an important role in facilitating proximal tubule bicarbonate reabsorption, as evidenced by a 90% inhibition in bicarbonate reabsorption with carbonic anhydrase inhibitors [49]. A paucity of carbonic anhydrase in neonatal proximal tubules would result in lower rates of bicarbonate reabsorption. There are significant species differences in the renal maturation of carbonic anhydrase [50]. The fetal rhesus monkey has significantly less renal carbonic anhydrase activity than the neonate [51]. In the rabbit, monkey and rat there is significant postnatal renal maturation [51–54]. In the rabbit the maturation of renal carbonic anhydrase isoforms have been studied. Type II carbonic anhydrase, the cytosolic isoform which comprises over 90% of total activity, increases twofold during postnatal renal maturation [52]. In addition, while in four-week-old and adult animals carbonic anhydrase II activity increases with metabolic acidosis, this does not appear to be the case for the neonate. The membrane bound carbonic anhydrase IV comprises only ~5% of renal carbonic anhydrase activity. Preliminary studies suggest that there is significant renal maturation of this isoform which may play an important role in the maturation of proximal tubule acidification [54]. Carbonic anhydrase may not be a factor in the maturation of human renal bicarbonate reabsorption, where levels in neonates are comparable to adults [55–57]. Carbonic anhydrase staining is detectable in proximal tubules at 12 to 15 weeks gestation in the human [57]. While there is little staining in the nephrogenic zone, proximal tubules from human fetus at 24 weeks gestation resemble that of the adult segment.

Maturation of Na^+/H^+ antiporter isoforms

Several isoforms of the Na^+/H^+ antiporter have been cloned [58–65]. Two isoforms of the Na^+/H^+ antiporter, NHE-1 and NHE-3, have been localized to the proximal tubule [66–69]. NHE-1, the housekeeping Na^+/H^+ exchanger, has a wide distribution in mammalian tissues and is found on the basolateral membrane of the proximal tubule [67]. There is increasing evidence that NHE-3 is an important isoform mediating proton secretion in the proximal convoluted tubule. NHE-3 encodes an amiloride-insensitive antiporter [63] whose activity increases with both acidosis and administration of glucocorticoids [70–72].

Recently, we characterized the isoforms of the Na^+/H^+ antiporter which increase with rabbit proximal tubule maturation [73]. The maturational changes of NHE-3 and NHE-1 mRNA were compared with β-actin and are shown in Figure 3. NHE-3 mRNA abundance was extremely low at one day of age and increased significantly in the first week of life. There was a significant increase in renal cortical NHE-3 mRNA abundance with postnatal maturation. NHE-3 mRNA abundance in one-week-old neonates was approximately one-fourth that of adult animals. The maturational changes in NHE-3 mRNA abundance was very similar to the maturational changes in Na^+/H^+ antiporter activity previously described. In contrast, there was no change in NHE-1 mRNA abundance with postnatal maturation. We have also examined the maturational changes of NHE-2 and NHE-4 mRNA [74]. NHE-2, like NHE-1, did not change significantly with postnatal maturation. NHE-4 mRNA is not detectable in the renal cortex at any stage of postnatal maturation.

To examine the postnatal maturation of Na^+/H^+ protein abundance, SDS-PAGE and immunoblotting was performed using polyclonal antibodies to NHE-3 and NHE-1 [73]. As shown in Figure 4, the maturational changes in NHE-3 and NHE-1 protein abundance was similar to that for NHE-3 and NHE-1 mRNA abundance. Again, NHE-3 protein abundance in one-week-old rabbits was one-fourth that of adults and fairly constant for the first two weeks of life. Adult levels of NHE-3 protein abundance were present at six weeks of age. NHE-1 protein abundance did not change significantly with postnatal maturation. These data provide further evidence that NHE-3 is an important isoform responsible for proton secretion in the proximal tubule and that

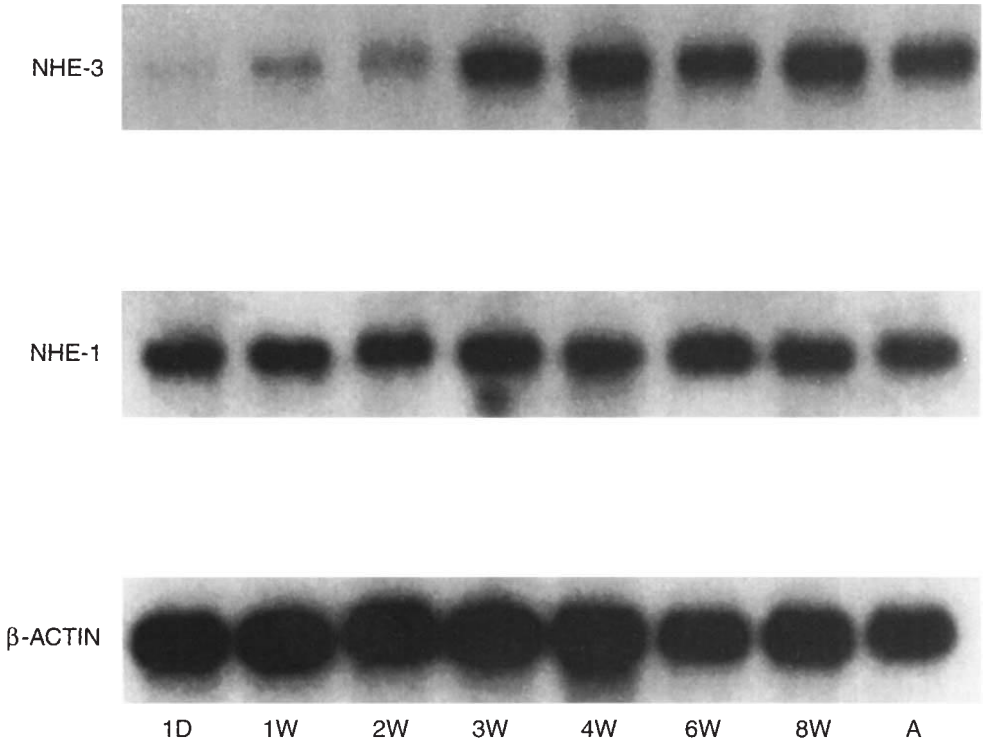

Fig. 3. *Maturational changes in NHE-3, NHE-1 and β-actin mRNA abundance.* Five micrograms of poly (A^+) RNA from renal cortex of one-day-old, and 1, 2, 3, 4, 6, and 8-week-old rabbits as well as adult rabbits, were isolated, fractionated and assayed for NHE-3, NHE-1 and β-actin mRNA abundance using Northern blot analysis. There was a significant increase in NHE-3 mRNA abundance with postnatal maturation ($P < 0.05$), but no change in NHE-1 mRNA abundance. From [73] with permission from the *American Journal of Physiology*.

there is a maturational parallel increase in apical Na^+/H^+ antiporter activity, NHE-3 mRNA levels, and NHE-3 protein abundance.

The issue of the polarity of NHE-3 and NHE-1 in the neonatal proximal tubule has not been resolved. While NHE-3 is located on the apical membrane [66] and NHE-1 is solely on the basolateral membrane of the adult proximal tubules [67], this has not been demonstrated in neonatal proximal tubules. The lack of polarity of these isoforms could be a factor in the lower rate of proximal tubular transport.

Role of glucocorticoids in proximal tubule maturation

The factors responsible for the postnatal maturational changes in proximal tubule acidification are unknown. There are significant postnatal maturational changes in the concentration of several hormones, including thyroid hormone and glucocorticoids [75–78]. These hormones have been shown to affect the maturation of several other organs including the liver, intestine and lung [79–81]. In addition, there may be postnatal maturational changes in other hormones and growth factors which could play an important role but have not yet been characterized. Finally, there is a dramatic increase in the glomerular filtration rate in both juxtamedullary and superficial nephrons, which by increasing sodium delivery to the proximal tubule, may cause a maturation of the transporters on the apical membrane [15, 82].

The best studied factors thought to play an important role in postnatal maturation of the proximal tubule are glucocorticoids. Serum glucocorticoids increase with postnatal maturation and administration of glucocorticoids increase proximal tubule Na^+,K^+-ATPase activity [41, 42]. In addition to potentially being a factor to explain the postnatal maturational increase in proximal tubular transport, they may be a factor to increase glomerular filtration rate with maturation and could explain the maturational decrease in proximal tubule phosphate transport [83].

The mechanisms whereby glucocorticoids affect proximal tubule acidification has recently been clarified. While it has long been known that systemic administration of glucocorticoids to adult animals produces a profound increase in Na^+/H^+ antiporter activity in adult animals [84–86], these effects could potentially be explained by an augmentation in glomerular filtration rate. However, several recent studies have directly demonstrated an epithelial action of glucocorticoids on the proximal tubule. In studies using *in vitro* microperfusion, the addition of 10^{-6} or 10^{-5} M dexamethasone to the bathing solution resulted in a 30% stimulation in the rate of bicarbonate absorption in rabbit proximal tubules after a three hour incubation [87]. Aldosterone had no effect on bicarbonate absorption. The dexamethasone induced stimulation of proximal acidification was blocked by actinomycin D and cycloheximide. Thus, the direct effect of glucocorticoids on proximal tubule bicarbonate absorption was dependent upon RNA and protein synthesis.

To directly examine whether glucocorticoids can increase Na^+/H^+ antiporter activity, we examined the effect of dexamethasone on OKP cells, a cell line with many characteristics of proximal tubule cells [88]. Dexamethasone induced a dose and time dependent stimulation of Na^+/H^+ antiporter activity. The

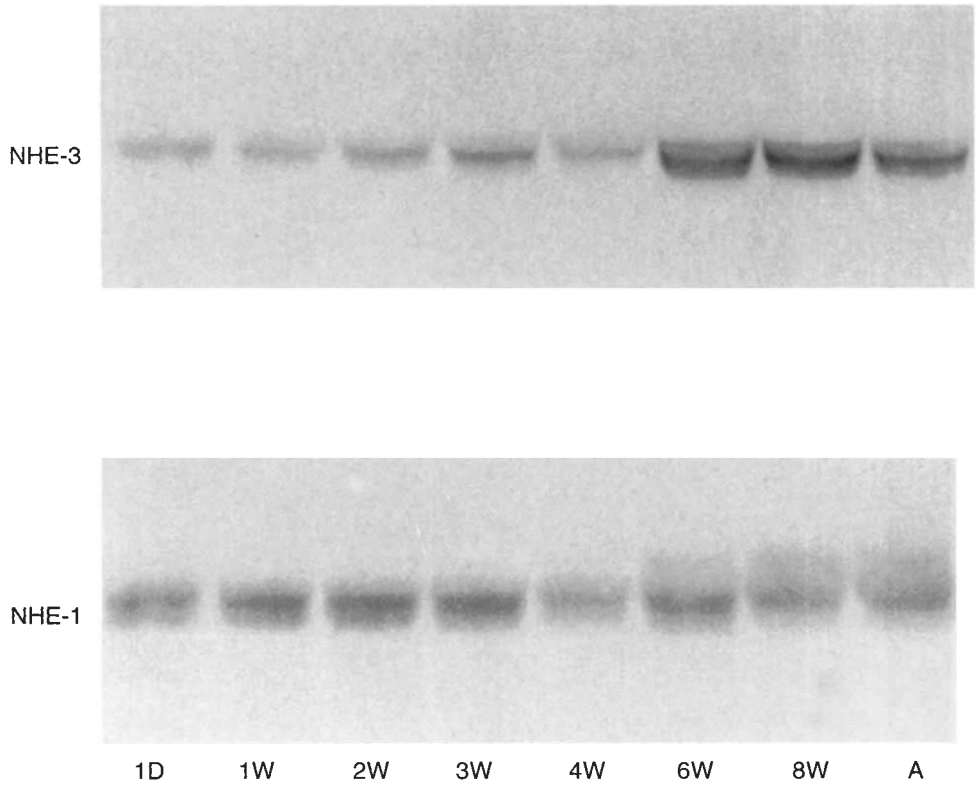

Fig. 4. *Immunoblot of rabbit renal cortical NHE-3 and NHE-1.* A total of 100 μg of renal cortical membranes from one-day-old, and 1, 2, 3, 4, 6, and 8-week-old and adult rabbits were fractionated using SDS-PAGE. There was a significant increase in NHE-3 protein abundance with maturation, ($P < 0.01$), but no change in NHE-1 protein abundance. From [73] with permission from the *American Journal of Physiology*.

effect of dexamethasone was entirely due to an increase in V_{max} without any change in the $K_{m(Na)}$. Again, the stimulatory effect of dexamethasone was dependent upon protein synthesis and was not seen with comparable doses of aldosterone. In addition to these findings, a direct effect of glucocorticoids on rabbit proximal tubule Na^+/H^+ antiporter activity has also been demonstrated in tubule suspensions [71, 89]. Therefore, in addition to the potential effect of glucocorticoids on renal hemodynamics, glucocorticoids directly affect proximal tubule transport and increase Na^+/H^+ antiporter activity.

The Na^+/H^+ antiporter activity in late fetal New Zealand White rabbits is approximately 25% of that measured in the adult. Administration of low doses of glucocorticoids (5 μg/100 g body wt) to pregnant does two days prior to delivery increased the V_{max} of the antiporter to levels seen in the adult animal [32]. The $K_{m(Na)}$ was unaffected. This effect was specific for the Na^+/H^+ exchanger as Na^+-glucose cotransport was not changed by glucocorticoid administration.

Similar results were found in isolated perfused tubules from pregnant New Zealand White rabbits given dexamethasone (6 μg/100 body wt) for the three days prior to the expected date of delivery [90]. Dexamethasone treated rabbits studied within 48 hours of birth had an almost twofold increase in the rate of bicarbonate absorption as compared to vehicle-treated control animals. The rate of bicarbonate absorption in dexamethasone treated neonates was comparable to what we measure in adult rabbits in our laboratory. In addition, the rate of Na^+/H^+ antiporter activity in juxtamedullary proximal convoluted tubules increased over twofold and the rate of $Na(HCO_3)_3$ symporter activity increased twofold in dexamethasone treated neonates [90].

We have recently examined the effect of glucocorticoids on NHE-3 and NHE-1 mRNA and protein abundance to determine which isoform of the Na^+/H^+ antiporter was affected by glucocorticoids [73]. As shown in Figure 5, administration of low doses of dexamethasone to neonates resulted in a twofold increase in NHE-3 mRNA abundance. Similar results were obtained when fetal rabbits received glucocorticoids late in gestation. There was no change in NHE-1 mRNA abundance. The results examining the effect of glucocorticoids on neonatal NHE-3 protein abundance using SDS-PAGE was even more pronounced. NHE-3 protein abundance increased to levels comparable to that seen in the adult renal cortex. Again, there was no significant change in NHE-1 protein abundance. These data demonstrate that glucocorticoids have a profound effect on renal acidification in neonates and that the effect of glucocorticoids is specific for the NHE-3 isoform.

In summary, the demands of the neonatal proximal tubule to reabsorb the majority of filtered solutes including bicarbonate are quite comparable to that of the adult nephron segment. There are maturational changes in the transporters responsible for active bicarbonate reabsorption. Glucocorticoids may play an important role in the postnatal maturational changes in proximal tubule solute transport. Future studies will need to clarify other factors

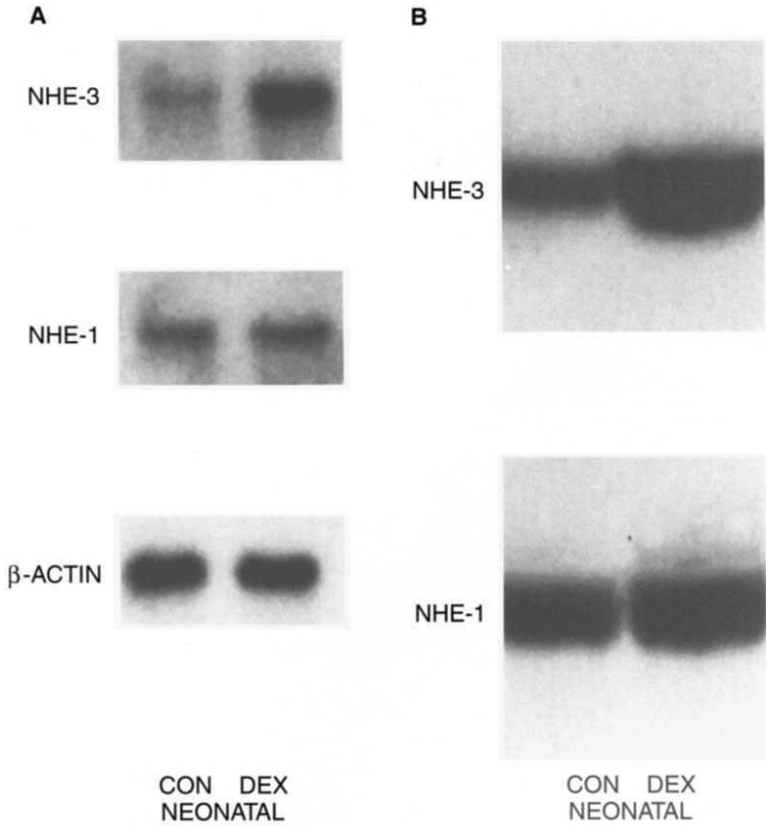

Fig. 5. a. *Effect of dexamethasone on NHE-3, NHE-1 and β-actin mRNA abundance.* Neonatal rabbits received vehicle or 10 μg/100 g dexamethasone for three days and two hours before sacrifice. There was a significant increase in NHE-3 ($P < 0.05$) but no change in NHE-1 mRNA abundance. **b.** Effect of dexamethasone on NHE-1 and NHE-3 protein abundance in renal cortical membranes. A total of 100 μg membranes from vehicle treated control neonates and neonates which received dexamethasone. There was no difference in NHE-1 protein abundance, but a significant increase in NHE-3 to adult levels. From [73], used with permission from the *American Journal of Physiology*.

that are responsible for proximal tubular maturation, and also how the different peritubular and hormonal factors which regulate adult proximal tubule acidification affect transport in the neonate.

Michel Baum and Raymond Quigley
University of Texas Southwestern Medical Center, Dallas, Texas, USA

Reprint requests to Michel Baum, M.D., Department of Pediatrics, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, Texas 75235-9063, USA.

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